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Differential Foliar Sensitivity of Soybean Cultivars to Ozone Associated with Differential Enzyme Activities

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Abstract

Soybean [*Glycine max* (L.) Merr.] cultivars Dare and Hood were exposed to ozone (980 $\mu g/m^3$) for 2 h to determine if differences in cultivar sensitivity were associated with differential activation of selected enzymes. The first trifoliate leaves of the cultivars were in similar stages of development (14 days from sowing) when exposed. The ozone treatment increased the activities of glucose 6-phosphate dehydrogenase, phenylalanine ammonia lyase, polyphenol oxidase and peroxidase above the control in both cultivars within 30 h after exposure. However, the activity of all these enzymes increased several hours sooner in Dare (sensitive) than in Hood (tolerant). Our data suggest that the differential sensitivity of these two cultivars to ozone could be based on the differential ozone induced activation of the enzymes involved in the lesion formation process.

Introduction

Cultivars of many plant species, including soybeans, exhibit a range in the amount of foliar chlorosis and necrosis resulting from an exposure to ozone (Brennan *et al.* 1964, Tingey *et al.* 1972, Howell *et al.* 1971, Reinert *et al.* 1972, Ormrod *et al.* 1971, Menser and Hodges 1970). This differential foliar sensitivity has been attributed to a variety of processes. Engle and Gabelman (1966) reported that the stomata of a tolerant onion cultivar closed more rapidly than those of a sensitive cultivar in the presence of ozone. A tolerant tobacco cultivar (Bel-B) exhibited a lower leaf water potential and a higher leaf resistance to gas exchange than Bel-W_3, an ozone sensitive cultivar (Ting and Dugger 1971). These changes in leaf water potential and leaf resistance could reduce foliar injury by restricting ozone diffusion into the leaf. Differences in tobacco cultivar sensitivity have also been associated with the foliar levels of soluble sugars (Dugger *et al.* 1966). Hanson *et al.* (1971) related foliar ascorbic acid levels to differential ozone tolerance of petunia cultivars. In contrast, Menser (1964) was unable to attribute differences in tobacco cultivar sensitivity to endogenous ascorbic acid levels. Howell (1974) has associated differential foliar sensitivity to differences in phenolic metabolism.

Plants subjected to biotic pathogens exhibit increases in enzyme activities and oxidative processes — for example, the activation of G6PD, PAL, PPO, and PRO — that lead to lesion formation and necrosis (Farkas and Solymosy 1965, Goodman *et al.* 1967). Tingey *et al.* (1975) found that an ozone exposure that induced foliar necrosis also increased the activities of G6PD, PAL, PPO, PRO. The association between enzyme activities increased by ozone, and lesion formation, is supported by the work of Koiwai and Kisaki (1973), in which inhibitors of mixed function oxidases protected plants against ozone injury.

The objective of this study was to determine if differences in soybean cultivar sensitivity based on foliar injury, could be explained by differential responses of enzymes involved in lesion formation.

Abbreviations: G6PD, Glucose 6-phosphate dehydrogenase; Phenylalanine ammonia lyase; PPO, Polyphenol oxidase; PRO, Peroxidase.

Methods and Materials

Soybeans [*Glycine max* (L.) Merr.] cv. Dare and Hood were grown and exposed in the facilities of the Southeast Plant Environmental Laboratories as previously described (Tingey *et al.* 1973). To determine if there were differences n leaf development between the two cultivars, leaf area, leaf fresh weight and relative stomatal resistance were determined on the first trifoliate leaves of plants ranging in age

from 8 to 24 days after sowing. The relative stomatal resistance was determined with an Alvim porometer as the time for the pressure to drop from 140 to 40 mmHg (Alvim 1966). A similar group of plants (8–24 days from sowing) were exposed to 980 $\mu g/m^3$ for 2 h to determine the growth stage of the first trifoliate leaf that exhibited the maximum ozone sensitivity. Foliar injury was visually assessed in 5% increments at 48 h following exposure.

Soybeans were exposed to either 0 or 980 $\mu g/m^3$ of ozone for 2 h when the trifoliate leaves were about 60 to 80% expanded, 14 days after sowing, the time of maximum ozone sensitivity (Figure 1). First trifoliate leaves were then visually examined and harvested at 0, 3, 6, 24, or 30 h after exposure for enzyme activity determinations. In all assays, the excised first trifoliate leaves were pulverized with a pestle in a mortar containing liquid nitrogen. The preparation of enzyme homogenates and the assay of the enzymes (G6PD, PAL, PPO and PRO) were conducted as previously described (Tingey *et al.* 1975).

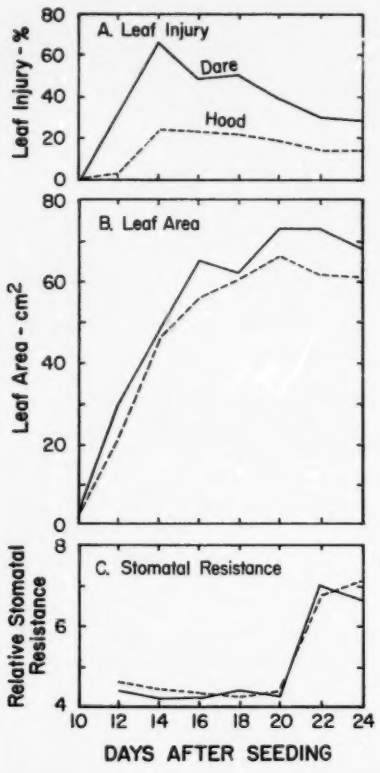

Figure 1. *Foliar injury, leaf growth, and relative stomatal resistance in soybean cultivars Dare and Hood.* Each mean is based on six observations, with an LSD (5%) of 14, 8.3, and 0.4, for the foliar injury, leaf area, and relative stomatal resistance, respectively. Dare = solid lines; Hood = dashed lines.

A replicated factorial design with duplicate plants was used for all experiments and data were subjected to analysis of variance. A LSD at the 5% significance level was used to compare differences among treatment means whenever a significant (5%) F statistic was found.

Results

Cultivar Dare exhibited significantly more foliar injury than Hood following an ozone exposure (Figure 1A). Foliar injury was not visible immediately after exposure but developed within 24 h and did not subsequently increase. For each cultivar, maximum sensitivity occurred 14 days after seeding when the leaf was 60 to 80% expanded (Figure 1B). Both Dare and Hood exhibited similar rates of leaf expansion as indicated by a nonsignificant F statistic for the cultivar by leaf age interaction. Leaf fresh weight followed a pattern similar to that of leaf area. The stomatal resistance of the 2 cultivars was not significantly different (Figure 1C), remaining low until the leaf was expanded (day 20) then increasing sharply. Maximum foliar injury, as expected, occurred during the period of low stomatal resistance. Foliar sensitivity to injury started to decrease prior to the sharp increase in leaf resistance to gas diffusion.

G6PD activities of the control plants were similar between the 2 cultivars and tended to increase over the 30 h study period (Figure 2A). Immediately after the ozone exposure the enzyme activities were the same for the treated and control plants of both cultivars. Within 3 h, G6PD activity in Dare was significantly above the control level and continued to increase for an additional 3 h. After that time, activity remained significantly above the control level for the remainder of the study. In contrast, G6PD activity in Hood was not significantly above the control until 6 h after exposure. The G6PD activity increased till 24 h before stabilizing above the control level. Dare maintained a higher level of post-exposure enzyme activity than Hood.

PAL activities of the control plants were similar for the cultivars and increased throughout the study period (Figure 2B). Immediately following the ozone exposure, PAL activity was depressed in Dare but not in Hood. PAL activity began to recover in Dare during the 3 h after exposure; within 6 h, PAL activity was 14% above the control level and remained significantly elevated for the rest of the study. After 6 h, PAL activity in Hood was 6% above the control and was significantly above the control after 24 h and 30 h. PAL activity tended to increase more rapidly in Dare than in Hood.

PPO activity was consistently higher in Dare than in Hood (Figure 2C), although PPO activity in the control plants of both cultivars decreased slightly during the study period. The ozone treatment significantly depressed PPO activity in both cultivars immediately after exposure, but more so in Dare (46%) than in Hood (12%). Within 3 h after exposure, PPO activity in Dare increased significantly

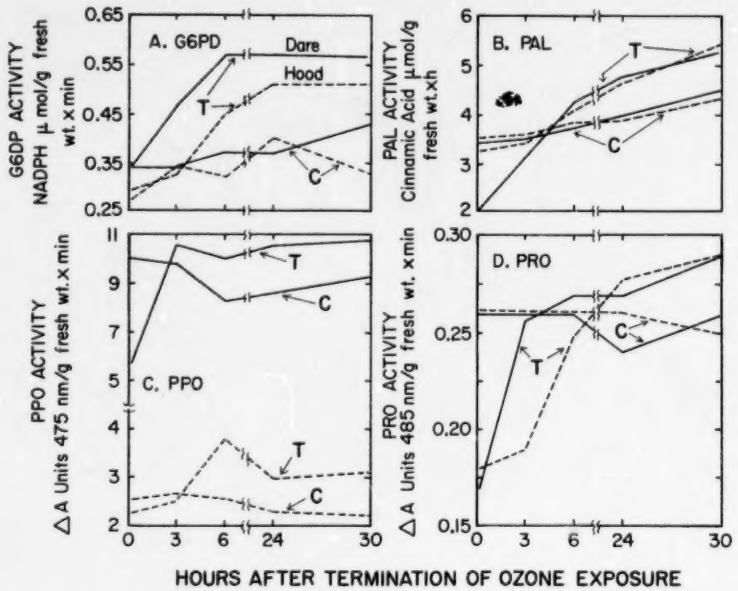
A. G6PD
Dare
Hood
T
C
G6DP ACTIVITY NADPH μ mol/g fresh wt. x min
B. PAL
PAL ACTIVITY Cinnamic Acid μmol/g fresh wt.xh
C. PPO
PPO ACTIVITY ΔA Units 475 nm/g fresh wt.x min
D. PRO
PRO ACTIVITY ΔA Units 485 nm/g fresh wt. x min
HOURS AFTER TERMINATION OF OZONE EXPOSURE

Figure 2. *Changes in selected enzymes in the first trifoliate leaf of soybean cultivars Dare and Hood following exposure to 980 μg/m³ ozone for 2 h.* The plants were exposed to ozone 14 days after sowing when the first trifoliate leaf was in a period of maximum sensitivity. Means are based on six observations with LSD (5%) of 0.05, 0.32, 0.29 and 0.02 for G6PD, PAL, PPO, and PRO, respectively. Dare = solid lines; Hood = dashed lines; A = absorbance; C = control; T = treatment.

above the control and remained above the control for the remainder of the study. In Hood, the increased PPO activity was not significantly above the control until 6 h after exposure, after which it remained elevated for the remainder of the study.

PRO activity was similar for the control plants of the two cultivars during the study period (Figure 2D). Immediately following the ozone exposure the enzyme activities in both cultivars decreased significantly. In cultivar Dare, the enzyme activity returned to the control level within 3 h and was significantly greater than the control level by 24 h following exposure. In contrast, PRO activity in Hood did not return to the control level until 6 h after exposure and was not significantly greater than the control until 30 h following exposure.

Discussion

The differential cultivar sensitivity, based on visual injury, observed for Dare and Hood was similar to a previous report (Tingey *et al.* 1973). Both cultivars exhibited similar characteristics of leaf growth and stomatal resistance. Maximum foliar injury for both cultivars occurred during the same stage of development (60 to 80% expanded), similar to previous reports (Ting and Mukerji 1971, Tingey *et al.* 1973). At the time of exposure, both cultivars exhibited a similar degree of stomatal resistance indicating that gas exchange was not a limiting factor in plant injury as it was for tobacco and onion cultivars (Ting and Dugger 1971, Engle and Gabelman 1966). The reported data indicated that the two soybean cultivars were in the same morphological and physiological stage at the time of the exposure.

The ozone induced increase in the activity of G6PD, PAL, PPO and PRO in the first trifoliate leaf of Dare within 24 h is similar to an earlier report (Tingey *et al.* 1975). The present study showed that G6PD, PAL, and PPO activities increased in both Dare and Hood within 3 to 6 h after the ozone exposure. These increases in enzyme activity occurred prior to the appearance of chlorotic and necrotic flecking on the upper leaf surface. However, ozone-induced pigment changes have been reported in ethanol-cleared leaves within 2 to 4 h following ozone treatment (Howell and Kremer 1973, Howell 1974). This time course for the appearance of ozone-induced pigments was similar to the time course for the activation of G6PD and PPO.

Howell and Kremer (1973) have identified some of the pigments formed in ozone-exposed leaves as polymerized phenols, proteins and amino acids. These workers suggested that the ozone induces changes that either allows more oxygen to enter the cells or allows oxidative enzymes to react with phenols, thereby forming quinones which poly-

merized with proteins and amino acids to form the pigments seen in injured tissue. Our data suggest that the phenols were initially oxidized through the action of PPO and later PRO. The involvement of PPO in lesion formation is supported by our observation that Dare, the more sensitive cultivar, has a higher level of enzyme activity than Hood. Furthermore, inhibitors of mixed function oxidases protect plants against ozone injury (Koiwai and Kisaki 1973). The increase in oxidative enzyme activities could indicate either an increased rate of protein synthesis or a decreased rate of protein removal.

In plants subjected to biotic pathogens, lesion formation is associated with an increase in the activities of G6PD, PAL, PPO and PRO resulting in the oxidation of phenols to quinones. The polymerized products are responsible for lesion formation (Farkas and Solymosy 1965, Goodman *et al.* 1967). Frequently cultivars resistant to pathogens exhibit a hypersensitive reaction and form lesions more rapidly than susceptible ones; thus the advance of the biotic pathogen is restricted. This response occurs because the enzymes involved in lesion formation are either activated sooner or reach a higher level in the resistant than in the susceptible cultivars. Howell (1974) has shown that sensitive peanut cultivars accumulate higher levels of phenols following ozone exposure than tolerant ones. The ozone treatment increased the activities of G6PD, PAL, PPO and PRO in Dare (sensitive) several hours before they were increased in Hood (tolerant). This difference in the rate of enzyme activation could result in more visual lesions in Dare than in Hood.

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